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Paracrine signalling between adipose tissue stem and microvascular endothelial cells within multilayered capsules trigger osteoblastogenesis

Introduction

Bone regeneration remains a challenging milestone in orthopedic research. Inspired by the synergic angio-osteogenesis, resulting from the co-existence of vascular and stem cells in the native environment of cancellous bone[1], we propose a stem-cell based strategy that provides an immunoprivileged atmosphere to induce osteoblastogenesis. We developed a hierarchical system to create a bottom-up tissue engineering (TE) approach combining *(i)* microparticles as cell adhesion sites (figure 1.A), and *(ii)* cells of different phenotypes (figure 1.B), encapsulated in *(iii)* multilayered liquified capsules (figure 1.C). Our hypothesis is that in the capsules confined and controlled environment, the paracrine interaction of co-cultured endothelial and human adipose-derived stem cells (hASCs) would trigger osteogenic differentiation and the generation of osteoblasts.

Materials and Methods

Liquified multilayered poly(L-lysine)-alginate-chitosan capsules containing collagen I functionalized PLLA microparticles, produced as previously described[2], were used to encapsulate hASCs alone or co-cultured with human adipose microvascular endothelial cells (CO capsules, 1:1). Capsules were maintained in EndoGro^{TM}-MV-VEGF medium (Millipore) without (basal medium) and with 10^{-8}M dexamethasone, 50µg.mL^{-1} ascorbic acid, and 10mM β-glycerophosphate (osteogenic medium) up to 21 days. Osteoblastogenesis was investigated by determining alkaline phosphatase (ALP) activity, and osteopontin secretion by immunocytochemistry. Statistical analysis was performed by two-way ANOVA with Bonferroni post-test.

Results

After 21 days in basal medium, the osteogenic phenotype of the cells in CO capsules was confirmed by the osteopontin expression (figure 1.D). At earlier culture periods (figure 1.E), the co-culture effect was less efficient on promoting osteoblastogenesis, compared to the effect promoted by osteogenic medium. Accordingly, at day 3 and 7, encapsulated cells in the capsules cultured in osteogenic medium presented higher ALP activity than in basal medium. However, at later culture periods, the ALP activity of the cells in CO capsules cultured in basal and osteogenic media was equivalent. Ultimately, at day 21, cells in the CO capsules cultured in basal medium presented the highest ALP activity. In opposition, hASCs in the homotypic capsules cultured in basal medium, used as control, presented very low ALP activity.

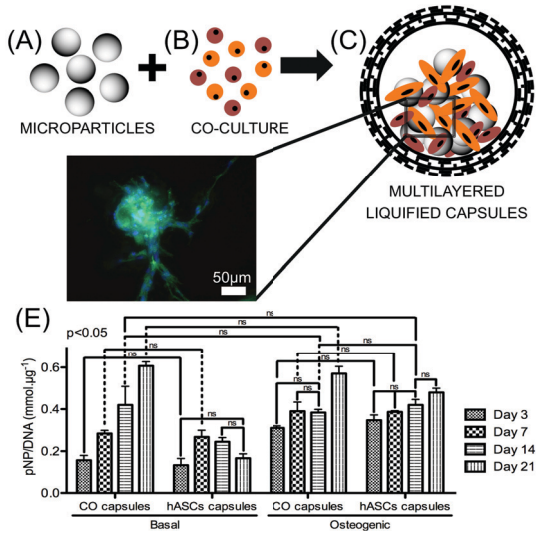

Figure 1 – (A) Microparticles and **(B)** cells of different phenotypes within **(C)** liquified multilayered capsules. **(D)** Aggregate of microparticles and cells, encapsulated in CO capsules after 21 days in basal medium, expressing osteopontin (green). Cells nuclei by DAPI counterstain (blue). **(E)** Alkaline phosphatase activity in CO and hASCs capsules at 3, 7, 14, and 21 days of culture in basal or osteogenic medium. $p<0.05$ unless otherwise specified (no statistically significant, ns).

Discussion and Conclusions

In the absence of osteogenic differentiation factors, the proposed co-culturing strategy supported osteoblastogenesis. Moreover, the developed system can be transplanted by minimal invasive procedures. Capsules can be directly injected into the lesion site due to the flexible multilayered membrane. We believe that our preliminary studies provide new insight into the importance of co-culture systems in engineering bioencapsulation systems for bone TE.

References

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Disclosures

Authors have nothing to disclose.